云南植物研究 2000，22（1）：103～108
Acta Botanica Yunnanica

圆果雪胆中的皂甙成分*

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摘要：从圆果雪胆（*Hemsleya amabilis*）的块茎中分离到 10 个化合物，其中 3 个雪胆皂甙是首次从该种植物中得到，它们的结构通过光谱和化学的方法鉴定为齐墩果酸－3－O－α－吡喃阿拉伯糖（1→3）－β－葡萄糖醛酸甙，28－O－β－D－吡喃葡萄糖齐墩果酸 3－O－α－吡喃阿拉伯糖（1→3）－β－D－葡萄糖醛酸，28－O－β－D－吡喃葡萄糖齐墩果酸 3－O－［β－D－吡喃葡萄糖（1→2）］－［α－吡喃阿拉伯糖（1→3）］－β－D－葡萄糖醛酸。同时还得到雪胆甲素，雪胆乙素，雪胆乙素甙，齐墩果酸和 β－谷甾醇等。

关键词：圆果雪胆；葫芦科；雪胆皂甙；雪胆素
中图分类号：Q 946　　**文献标识码**：A　　**文章编号**：0253－2700（2000）01－0103－06

Saponins from *Hemsleya amabilis**

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Abstract: From the rhizomes of *Hemsleya amabilis*, three saponins of oleanolic acid were isolated, named hemslosides－Am1, －Ma1 and －Ma3. Their structures have been elucidated as 3－O－α－arabinopyranosyl (1→3)－β－glucuronide of oleanolic acid, 28－O－β－D－glucopyranosyl ester of oleanolic acid 3－O－α－arabinopyranosyl (1→3)－β－glucuronide and 28－O－β－D－glucopyranosyl ester of oleanolic acid 3－O－[β－glucopyranosyl(1→2)]－[α－arabinopyranosyl(1→3)]－β－glucuronide, respectively. In addition, cucurbitacins, cucurbitacin Ⅱ glycoside, oleanolic acid and β－sitosterol were obtained.

Key words: *Hemsleya amabilis*; Cucurbitaceae; Hemslosides－Am1, －Ma1, and －Ma3; Cucurbitacins

The genus *Hemsleya* has its distribution center in south－west China, particularly in Yunnan and Sichuan. Recently, 31 species of this genus have been discovered of which 20 are found in Yunnan. *Hemsleya amabilis* Diels is distributed in middle and west of Yunnan and Guangxi (Jiangsu Institute of Botany *et al*, 1988).

The plants of this genus are known as herbal medicines in China, used in the treatment of bacillary dysentery, bronchitis and tuberculosis. Previously, isolation of dihydrocucurbitacin F (2) and its 25－O－acetate (1) from *H. amabilis* (Chen *et al*, 1975) and oleanane－type

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* Foundation item: Program granted by the Natural Sciences Foundation of Yunnan Province
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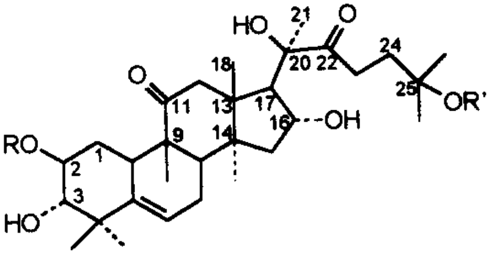
Received date: 1999－01－22, Accpted date: 1999－07－08

triterpenes from *H. mitrata* (Meng *et al*, 1985) were reported. Pursuing these studies, the present paper deals with the isolation and structure determination of cucurbitacins and hemslosides from *Hemsleya amabilis*.

Results and Discussion

The ethanolic extract of rhizomes of *H. amabilis* was suspended in H_2O, and extracted with EtOAc and n−butanol saturated with water, successively. From the EtOAc layer, seven compounds were isolated. Three of which were identified as 23,24−dihydrocucurbitacin F−25−acetate (1), 23,24−dihydrocucurbitacin F (2) (Meng *et al*, 1985), 23,24−dihydrocucurbitacin F 2−O−(−D−glucopyranoside (3) (Rui et al, 1985) by mixed melting point and comparison of MS, IR, ^{1}H and ^{13}C NMR spectra with those of authentic samples. In addition, (−sitosterol(4), oleanolic acid(5), octadecanoic acid(6) and methyl gadoleate (7) have been isolated.



	R	R'
1	H	Ac
2	H	H
3	Glc	H

The butanol layer of *Hemsleya amabilis* afforded three saponins (8−10). They were identified as hemslosides——Am1 (8), Mal (9) and −Ma3 (10). (Morita *et al*, 1986; Kasal *et al*, 1990).

Compound 8, called hemsloside−Am1, afforded D−glucuronic acid and L−arabinose on acid hydrolysis. Its negative FAB mass spectrum exhibited $[M-1]^-$ ion peak at m/z 763 and the fragment ions at m/z 631 [M−1−ara], and 455 [M−1−ara−glcUA]. This suggested that the sugar sequence of 8 was −glcUA−ara. The ^{13}C NMR spectrum (see Tables 1 and 2) exhibited two anomeric carbon signals (δ106.8, 106.1). By comparison of the ^{13}C NMR spectrum of **8** with that of oleanolic acid (5), the signal of C−2 was displaced upfield 1.8ppm, while the signal C−3 was shifted downfield 11.0ppm, which indicated that 8 is a 3−O−glycosylated oleanolic acid. The anomeric proton signals of 8 at δ 5.36 (1H, d, J=7.6Hz), 4.98 (1H, d, J=7.96Hz) as well as the anomeric carbon signals of 8 indicated that the anomeric configuration of their glucuronide and arabinoside linkages to be β and α, respectively. Thus, compound **8** can be formulated as 3−O−α−arabinopyranosyl (1→3)−β−glucuronide of oleanolic acid.

Compound 9 was assigned the molecular formula $C_{47}H_{74}O_{18}$ by the negative ion FAB−MS, in which a $[M-1]^-$ ion peak was exhibited at m/z 925 and the fragment ion peaks at m/z 763 [M−1−glc], 793 [M−1−ara], 631 [M−1−glc−ara], 455 [M−1−glc−ara−glcUA]. By comparison of ^{13}C NMR spectra with those of 8, 9 showed a set of additional signals of a terminal glucopyranosyl unit which was proved to be β−glucosyl ester of 5 (oleanoic acid) (see Table 1).

The anomeric proton signals at δ6.32(1H, d, J=8Hz), 5.37(1H, d, J=7.36Hz) and

4.97（1H，d，J = 8. 36Hz） were consistent with these configuration. From these results，compound 9 was confirmed as 28 – O – β – D – glucopyranosyl ester of oleanolic acid 3 – O – α – arabinopyranosyl （1→3） – β – glucuronide. Its ^{1}H and ^{13}C – NMR spectra were identical with those of authentic sample （Nie *et al*，1984；Shi *et al*，1991）.

Similarly，by comparison of ^{13}C NMR spectrum with those of **9**，**10** only showed a set of additional signals of β – D – glucopyranosyl unit which was attached to C – 2 of the glucuronic acidof 9. For the C （2） signal of glucuronic acid was obviously shifted downfield 5ppm. It was assigned the molecular formula $C_{53}H_{84}O_{23}$ by the negative ion FAB – MS，in which a molecular ion peak was exhibited at m/z 1088. Thus，the structure of **10** was assigned as 28 – O – β – D – glucopyranosyl ester of oleanolic acid 3 – O – [β – glucopyranosyl（1 → 2）] – [α – arabinopyranosyl（1→3）] – β – glucuronide. Its 1H and ^{13}C NMR spectra were identical with those of authentic sample （Nie *et al*，1984；Shi *et al*，1991）.

Table 1 ^{13}C NMR data for the aglycone moieties of 5，8，9 and 10 in C_5D_5N

	5	8	9	10
C – 1	38.9	38.8	38.7	38.7
C – 2	28.2	26.4	26.5	26.5
C – 3	78.0	89.6	89.3	89.7
C – 4	39.4	39.4	39.5	39.6
C – 5	55.8	55.8	55.8	55.6
C – 6	18.8	18.6	18.6	18.5
C – 7	33.3	33.2	33.1	33.1
C – 8	39.8	39.5	40.0	40.0
C – 9	48.1	48.1	48.1	48.1
C – 10	37.4	37.2	37.0	37.0
C – 11	23.8	23.8	23.5	23.5
C – 12	122.5	122.9	122.9	122.9
C – 13	144.8	144.8	146.4	144.1
C – 14	42.0	42.0	42.2	42.2
C – 15	28.3	28.3	28.3	28.3
C – 16	23.8	23.8	23.7	23.8
C – 17	46.7	47.1	47.1	47.1
C – 18	42.0	42.0	41.6	41.8
C – 19	46.7	46.3	46.3	46.3
C – 20	31.0	30.9	30.8	30.8
C – 21	34.3	34.2	33.1	34.1
C – 22	33.3	33.2	32.6	32.6
C – 23	28.7	28.1	28.3	28.0
C – 24	16.5	16.9	16.9	16.7
C – 25	15.5	15.4	15.5	15.5
C – 26	17.5	17.4	17.5	17.5
C – 27	26.2	26.2	26.1	26.1
C – 28	180.2	180.2	176.4	176.4
C – 29	33.3	33.2	33.1	33.1
C – 30	23.8	23.8	23.7	23.7

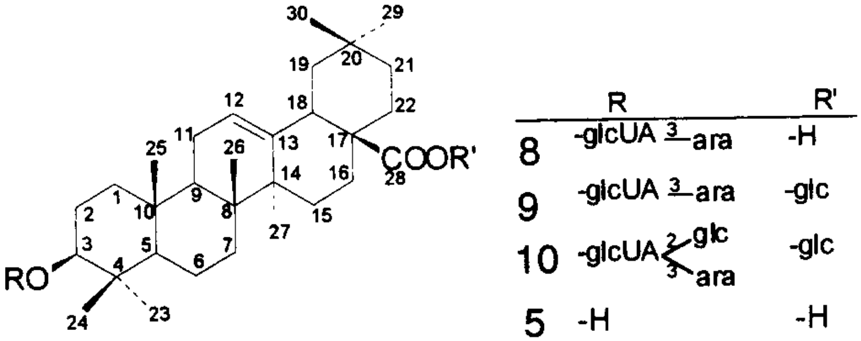
Table 2 ^{13}C NMR data for the sugar moieties of 8，9 and 10 in C_5D_5N

	8	9	10
3 – GlcUA 1	106.1	105.7	105.2$^{a)}$
2	74.4	74.4$^{a)}$	79.2
3	86.1	85.9	86.3
4	72.7	72.7	72.9
5	77.4	77.4	77.3
6	172.1	172.0	171.7
Ara 1	106.8	106.5	105.4$^{a)}$
2	71.4	71.4	71.5
3	74.4	74.7$^{a)}$	74.7
4	69.4	69.2	69.6
5	67.1	67.1	67.8
Glc 1	–	–	103.8$^{a)}$
2	–	–	76.4
3	–	–	78.9$^{b)}$
4	–	–	72.6
5	–	–	77.7$^{b)}$
6	–	–	63.4
28 – Glc 1	–	95.8	95.8
2	–	74.2	74.2
3	–	79.2$^{b)}$	78.9$^{b)}$
4	–	71.3	71.3
5	–	78.9$^{b)}$	79.2$^{b)}$
6	–	62.4	62.4

a） b）：Assignments in the vertical column with the sign may be alternated.

gluUA ：β – D – glucuronic acid，ara：α – L – arabinopyranosyl，glc：β – D – glucopyranosyl

These three saponins were also isolated from *H. macrosperma* （Nie *et al*，1984） and *H. graciliflora* （Kasal *et al*，1990）.



	R	R'
8	-glcUA $\xrightarrow{3}$ ara	-H
9	-glcUA $\xrightarrow{3}$ ara	-glc
10	-glcUA $<^{2}$ glc ^{3} ara	-glc
5	-H	-H

Fig. 2 Chemical structure of hemslosides

Experimental

General Procedure Mps. uncorrected. Optical rotations were measured on a J－20C polarimeter. NMR spectra were recorded with a Bruker AM－400 MHz instrument，using TMS as an internal standard. FAB－MS spectra were measured with VG Auto Spec－3000 spectrometer. IR spectra were measured on Bio－Rad FTS 135 instrument in KBr pellets. CC were carried out with silica gel （Marine Chemical Industry Factory，Qingdao），and Lichroprep RP－8 （Merck，Co. Ltd，germany）. The spots were visualized by spraying with 20% H_2SO_4 followed by heating.

Plant material *Hemsleya amabilis* was collected at Songming，Yunnan，China. Specimen was taxonomically identified by Cheng－Yih Wu and deposited in the Herbarium of Kunming Institute of Botany，the Chinese Academy of Sciences. Extraction and separation The dried and powdered rhizomes （7.8 kg） were extracted with hot 95% EtOH for three times. The extract was evaporated to dryness in vacuo. A suspension of this extract （1.8 kg） in H_2O was extracted with EtOAc and n－BuOH saturated with H_2O，respectively. The EtOAc layer was concentrated to yield an EtOAc extract （404 g）. The BuOH layer was concentrated to dryness to give a crude saponin fraction （1 025 g）.

The EtOAc extract （404 g） was dissolved in water and was subjected to macroporous absorption resin D－101，eluting with aq. MeOH，to give five fractions，namely Fr－1 （eluting with H_2O），Fr－2 （eluting with 20% MeOH，5 g），Fr－3 （eluting with 50% MeOH，15 g），Fr－4 （eluting with 50% MeOH，5 g），Fr－5 （eluting with 80% MeOH，0.9 g）.

Fr－3 was repeatedly chromatographed on silica gel，eluting with $CHCl_3$－CH_3OH （9∶1） to give **2** （109 mg） and **3** （90 mg）. Fr－4 was repeatedly chromatographed on silica gel，eluting with petro.－acetone （9∶1 to 8∶2） to give **1** （76mg）. Fr－5 was repeatedly chromatographed on silica gel，eluting with $CHCl_3$－CH_3COCH_3 to give **4** （30 mg），**5** （20 mg），**6** （0.54 g），and **7** （20 mg）.

The n－BuOH extract was dissolved in MeOH，then poured into an excess of acetone to give a precipitate. The precipitate (10 g) was separated into three fractions (Fr－1－3) in the increasing polarity by column chromatography on silica gel eluting with $CHCl_3$－MeOH－H_2O (30∶10∶1－20∶10∶1－10∶5∶1).

Fr－1, after repeated chromatography on a reverse phase column of RP－8, eluting with acetone－H_2O (50%), then repeated column chromatography on silica gel [solvent: $CHCl_3$－MeOH－H_2O (7∶3∶1, lower layer)], followed by deionization with ion exchange resin (Amberlite MB－3), afforded 8 (23 mg) yield: 0.23%, white powder (from MeOH－H_2O), $C_{41}H_{64}O_{13}$, FAB－MS (negative) m/z (rel. int.): 763 $[M-1]^-$ (100), 631 [M－1－Ara] (18). ^1H NMR (C_5D_5N) δ: 5.36 (1H, d, J＝6.76Hz, glcUA H－1), 4.98 (1H, d, J＝7.96Hz, ara H－1). ^{13}C NMR data see Tables 1 and 2.

Fr－2, after repeated chromatography on a reverse phase column of RP－8, eluting with acetone－H_2O (50%), followed by deionization with ion exchange resin (Amberlite MB－3), afforded 9 (37 mg), yield: 0.37%, white powder (from MeOH－H_2O), $C_{47}H_{74}O_{18}$, FAB－MS (negative) m/z (rel. int.): 925 $[M-1]^-$ (100), 763 [M－1－glc] (28), 793 [M－1－ara] (15), 631 [M－1－glc－ara] (18), 455 [M－1－glc－ara]. ^1H NMR (C_5D_5N) δ: 6.32 (1H, d, J＝8.0Hz), 5.37 (1H, d, J＝7.36Hz, glcUA H－1), 4.97 (1H, d, J＝8.88Hz, ara H－1). ^{13}C NMR data see Tables 1 and 2.

Fr－3, after repeated chromatography on a reverse phase column of RP－8, eluting with acetone－H_2O (35∶65), followed by deionization with ion exchange resin (Amberlite MB－3), afforded **10** (67 mg) yield: 0.67%, colorless needles (from MeOH－H_2O), $C_{53}H_{84}O_{23}$, FAB－MS (negative) m/z (rel. int.): 1088 [M]－ (100), 925 [M－1－glc] (13). IR (KBr): cm^{-1} 3426, 2947, 1723, 1638, 1080, 1050. ^1H NMR (C_5D_5N) δ: 6.32 (1H, d, J＝8.4Hz), 5.70 (1H, d, J＝7.9Hz), 5.32 (1H, d, J＝7.6Hz, glcUA H－1), 4.96 (1H, d, J＝7.6Hz, ara H－1). ^{13}C NMR data see :Tables 1 and 2.

Acid hydrolysis of **8** was heated with 5% H_2SO_4 in H_2O for 10h at 70℃. The reaction mixture was tested by TLC technique, solvent: $CHCl_3$－MeOH－H_2O (30∶12∶4 lower layer 9mL ＋ acetic acid 1mL) (Zhao *et al*, 1987).

Acknowledgements We would like to thank the members of analytical group in the Phytochemistry Laboratory, Kunming Institute of Botany for their measuring the spectral data.

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